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Year: 1989

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DOI: <https://doi.org/10.1093/carcin/10.1.43>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-155233>

Journal Article

Published Version

Originally published at:

Hegi, Monika E; Sagelsdorff, Peter; Lutz, Werner K (1989). Detection by 32P-postlabeling of thymidine glycol in  $\gamma$ -irradiated DNA. *Carcinogenesis*, 10(1):43-47.

DOI: <https://doi.org/10.1093/carcin/10.1.43>

# Detection by <sup>32</sup>P-postlabeling of thymidine glycol in γ-irradiated DNA

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The <sup>32</sup>P-postlabeling method has been adapted for the analysis of thymidine-*cis*-glycol-3'-phosphate (*cis*-dTGP, *cis*-5,6-dihydroxy-5,6-dihydrothymidine-3'-phosphate). *Cis*-dTGP was isolated and purified from normal nucleotides by phenylboronate affinity chromatography and phosphorylated by T4 polynucleotide kinase in presence of 1 mM BeCl<sub>2</sub> at pH 7.5. These modifications of the postlabeling method resulted in a 5'-phosphorylation of dTGP with a labeling efficiency of up to 20% whereas the natural nucleotides were almost completely dephosphorylated at the 3' position under these conditions. The reaction products, containing radio-labeled thymidine-*cis*-glycol-3',5'-bis-[5'-<sup>32</sup>P]phosphate (*cis*-\*pdTGP), were separated by two-dimensional anion-exchange TLC on polyethyleneimine cellulose sheets. Boric acid was added in the second dimension in order to selectively retard *cis*-glycols. The method was applied to γ-irradiated nucleotides and calf thymus DNA. In the nucleotide mixture, 330–99 000 thymine glycol (TG) moieties were detected per 10<sup>6</sup> thymines (T) in a dose range of 14–1000 Gy respectively. In DNA, these values ranged from 400 to 2700 TG/10<sup>6</sup> T. The data are in good agreement with methods using radiochemical and immunological techniques. Non-irradiated DNA showed a background level of 10TG/10<sup>6</sup> T. This practical limit of detection was higher than can be achieved with the postlabeling technique, indicating that the present method might be a sensitive alternative for a determination of oxidative DNA damage.

## Introduction

Oxygen radicals such as the highly reactive hydroxyl radical are known to cause DNA damage (1). They are postulated to be partly responsible for the carcinogenic action of ionizing radiation. For a number of chemical carcinogens, an 'indirect' genotoxicity via the same ultimate reactive agent is also discussed (2). In these cases, the hydroxyl radical is thought to be generated by a Fenton reaction of superoxide anion radical O<sub>2</sub><sup>•</sup> with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Both reactants are produced in normal aerobic cellular metabolic pathways, but the concentration seems to be carefully controlled by the action of superoxide dismutase, peroxidases (including catalase) and antioxidants. Only in specific situations have increased levels of H<sub>2</sub>O<sub>2</sub> been shown

\*Abbreviations: TG, thymine glycol (5,6-dihydroxy-5,6-dihydrothymine); *cis*-dTGP, thymidine-*cis*-glycol-3'-phosphate (*cis*-5,6-dihydroxy-5,6-dihydrothymidine-3'-phosphate); PBA, phenylboronic acid; T, thymine; dTP, thymidine-3'-phosphate; dsDNA, double-stranded DNA; dNp, deoxyribonucleoside-3'-phosphate; LE, labeling efficiency; bicine, (*N,N*-bis[2-hydroxyethyl]glycine); TLC, thin-layer chromatography; \*p, [<sup>32</sup>P]phosphate; pdNp, deoxyribonucleoside-3',5'-bis-phosphate; pdN, deoxyribonucleoside-5'-phosphate; p<sub>i</sub>, inorganic phosphate; PNK, polynucleotide kinase.

to be produced, e.g. in liver peroxisomes from rodents treated with peroxisome proliferators (3,4) and in macrophages stimulated by phorbol ester tumor promoters (5). Under *in vitro* conditions, damage has been detected in DNA added extracellularly (3,5). However, no evidence for DNA damage *in vivo* has so far been reported.

In order to investigate whether intracellular DNA is also damaged under conditions of chemically induced oxygen stress, sensitive and specific methods for the detection of oxidative DNA damage are required. One important reaction product of DNA oxidation with hydroxyl radical is 5,6-dihydroxy-5,6-dihydrothymine (thymine glycol, TG\*). Two *cis*-isomers and two *trans*-isomers are formed (6). The present report describes a new method using a modified <sup>32</sup>P-postlabeling technique (7), which includes an affinity chromatography purification of dTGP and a phosphorylation procedure in the presence of mutagenic metal ions to achieve acceptable labeling efficiencies. The method is applied to quantitate oxidative DNA damage as *cis*-5,6-dihydroxy-5,6-dihydrothymidine-3'-phosphate (thymidine-*cis*-glycol-3'-phosphate *cis*-dTGP) in γ-irradiated DNA.

## Materials and methods

### Materials

Phenylboronate silica (Bondesil PBA) was obtained from Analytichem International (Basel, Switzerland). For PBA-chromatographies 0.7 × 20 cm Econo-columns were purchased from BioRad (Glattbrugg, Switzerland). Anion-exchange PEI–cellulose thin layer sheets were from Macherey-Nagel (Düren, FRG), Cawo cassettes and Cawo intensifying screens (fast tungstate) from Cawo (Zurich, Switzerland) and Kodak XAR-5 films from Kodak (Lausanne, Switzerland). Lyophilizations were performed in a speed vac concentrator from Bachhofer Laborgeräte (Reutlingen, FRG). The enzymatic digestion of DNA was carried out in Eppendorf tubes from Sarstedt AG (no. 72.690; Sevelen, Switzerland). All deoxyribonucleotides, thymidine, thymine (T) and ATP were purchased from Pharmacia (Dübendorf, Switzerland). Calf thymus DNA (no D-1501) and spermidine were obtained from Sigma and dithiothreitol from Serva Feinbiochemica (Heidelberg, FRG). [γ-<sup>32</sup>P]ATP (3000 Ci/mmol, NEG-002 H; 6000 Ci/mmol, NEG-002 Z for the experiments on the concentration dependence) was obtained from New England Nuclear. Potato apyrase (E.C. No. 3.6.1.5, grade I, ATPase activity 11 U/mg), micrococcal nuclease (E.C. No. 3.1.31.1, Sigma no. N3755) and nuclease P1 (E.C. No. 3.1.30.1) was obtained from Sigma, T4 polynucleotide kinase (E.C. No. 2.7.1.78) (10<sup>4</sup> U/ml) from Pharmacia, spleen exonuclease (= phosphodiesterase, E.C. No. 3.1.16.1, no. 108 251) and polynucleotide kinase, 3'-phosphatase-free (E.C. No. 2.7.1.78) from Boehringer Mannheim. All other chemicals were of the highest purity available from Merck or Fluka (Buchs, Switzerland).

### Synthesis of *cis*-dTGP

The synthesis of this standard essentially followed the published methods (8,9): 1 mg (3.1 μmol) thymidine-3'-phosphate (dTP) in 1 ml of 0.2 M NH<sub>4</sub>Cl/NH<sub>3</sub> buffer pH 8.6, was oxidized at 0°C with 0.4 ml 0.014 M KMnO<sub>4</sub> in the same buffer. After 5 min, 10 μl of 1 M Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub> was added to reduce the residual permanganate. MnO<sub>2</sub> was removed by centrifugation and 1.4 ml of 1 M NH<sub>4</sub>OAc/NH<sub>3</sub>, 40 mM MgCl<sub>2</sub> pH 8.8 (buffer A) was added to the supernatant. The sample was loaded onto a PBA affinity column (bed dimensions 0.7 × 2.5 cm; used at 4°C) equilibrated with 10 ml 0.5 M NH<sub>4</sub>OAc/NH<sub>3</sub>, 20 mM MgCl<sub>2</sub> pH 8.8 (buffer B). The column was washed with 15 ml buffer B and 5 ml 0.5 M NH<sub>4</sub>OAc/NH<sub>3</sub>, 1 mM MgCl<sub>2</sub> pH 8.8 (buffer C). dTGP was eluted with 50 mM HOAc and 0.5 ml fractions were collected. The amount of *cis*-dTGP formed was quantified by HI-reduction (8).

### γ-Irradiation

Double-stranded DNA (dsDNA), deoxyribonucleoside-3'-phosphate (dNp) or dTP

(all at 3.2  $\mu\text{mol}$  dNp/ml) were dissolved in bidistilled water and irradiated from a  $^{60}\text{Co}$  source at a dose rate of 46 Gy/min at room temperature.

#### Digestion of dsDNA to dNp

To 1 ml of the sample 250  $\mu\text{l}$  100 mM sodium succinate/HCl buffer, pH 6, containing 40 mM  $\text{CaCl}_2$  were added and the DNA was enzymatically digested for 16 h at 37°C with 2.5 U micrococcal nuclease added in 50  $\mu\text{l}$  8 mM  $\text{CaCl}_2$ , 20 mM sodium succinate, pH 6, and 0.05 U spleen exonuclease (in 12  $\mu\text{l}$  of the commercial suspension).

#### Separation of dTGp from normal nucleotides on a PBA column (at 4°C)

The digest (1300  $\mu\text{l}$ ) was mixed with 1300  $\mu\text{l}$  buffer A and loaded onto the phenylboronate affinity column (bed: 0.7  $\times$  2.5 cm) equilibrated with 10 ml buffer A. The chromatography was performed in the same way as described above (Synthesis of *cis*-dTGP). Fractions 2–8 were pooled and lyophilized to 1 ml. For further purification, the concentrated sample was loaded onto a second column after adding 1 ml buffer A and was treated as before. The samples were stored in Eppendorf tubes at –20°C either as 100- $\mu\text{l}$  aliquots of the fractions or in pools of 15  $\mu\text{l}$  of fractions 3–8 (= 90  $\mu\text{l}$ ). The samples were lyophilized immediately before phosphorylation.

To estimate the extent of normal dNp contaminating the dTGp fractions after two PBA columns, 3.2  $\mu\text{mol}$  dNp (equimolar for G, A, C and T) were chromatographed twice on PBA columns, 100- $\mu\text{l}$  aliquots of fractions 2–7 were taken, lyophilized and phosphorylated at pH 9.0 in presence of 50 pmol ATP [optimal phosphorylation conditions for dNp, resulting in a labeling efficiency (LE) of 80%].

#### Enzymatic phosphorylation of dNp with [ $\gamma$ - $^{32}\text{P}$ ]ATP

The following components were carefully placed as separate droplets on the inside wall of Eppendorf tubes: 2  $\mu\text{l}$   $\text{H}_2\text{O}$  (or 2  $\mu\text{l}$  of the different 5 mM metal ion solutions;  $\text{BeCl}_2/\text{HCl}$ , pH 3), 2  $\mu\text{l}$  kinase buffer (250 mM bicine/NaOH pH 6.0 to 9.0, 50 mM  $\text{MgCl}_2$ , 50 mM dithiothreitol, 5 mM spermidine), 2  $\mu\text{l}$  dNp solution (0.01–50 pmol) [ $\text{H}_2\text{O}$  or ATP solution (10–1000 pmol)] in those experiments where lyophilized samples were used], 2  $\mu\text{l}$  [ $\gamma$ - $^{32}\text{P}$ ]ATP (1  $\mu\text{Ci}$  to 45  $\mu\text{Ci}$ ). The four droplets were mixed by centrifugation. Two microliters (3 U) T4 PNK (freshly diluted in water) was placed on the inside wall and after a second centrifugation the tubes were incubated for 30 min at 37°C. The total volume of the sample was always 10  $\mu\text{l}$ . In order to hydrolyze unreacted ATP the mixture was incubated for another 15 min at 37°C with 40 mU (2  $\mu\text{l}$ ) potato apyrase (in water). The buffer used for the kinase reaction, bicine (*N,N*-bis[2-hydroxyethyl]glycine,  $\text{pK}_a$  8.3) was sometimes used below the ideal pH range because it was found that the LE for dTGp was higher with bicine even at low pH values compared with all other buffers tried (Hepes, imidazol, Tris, Tris-maleate).

#### Separation of $^{32}\text{P}$ -labeled nucleotides by thin-layer chromatography (TLC)

For the separation of [ $^{32}\text{P}$ ]pdTGp (\*pdTGp) from normal deoxyribonucleoside-3',5'-bis- $^{32}\text{P}$ ]phosphate (\*pdNp), a two-directional anion-exchange PEI–cellulose TLC system was developed (see Figure 1): The sheet (20  $\times$  20 cm, pre-washed by a run with bidistilled water and stored at –20°C when dry) was marked with a pencil to indicate the position of the sample (O), of the standards S1–S3 and the cutting line for S1 after the first dimension.

Up to 10  $\mu\text{l}$  of the phosphorylation mixture was applied to the origin (O) in the left and 2  $\mu\text{l}$  \*pdTGp standard (S1) was placed in the right lower corner. Without drying the origin area, direction 1 was developed in 0.12 M sodium phosphate pH 8.6 (~2 h). The sheet was dried in a stream of cold air, marked with 'ink dots' containing [ $^{32}\text{P}$ ]ATP for alignment after exposure (on the right side which is cut before dimension two), and autoradiographed for 10 min to localize origin and (inorganic [ $^{32}\text{P}$ ]phosphate)  $\text{P}_i$  spot. These areas were clipped off and kept for quantification by Cerenkov counting. The standard S1 was cut off at the right-hand side of the sheet. The sheet was washed in 500 ml deionized water in a flat tray (30  $\times$  40 cm) for 4 min. The tray was agitated frequently during washing. A 1-ml aliquot of the washing water was taken and counted for radioactivity. The sheet was again dried in a stream of cold air. For the second dimension the sheet was turned 90° counter-clockwise and two standards (S2 = \*pdTGp; S3 = \*pdTp) were applied on the new base line, 4 and 6 cm from the left corner respectively. The second dimension was developed in 0.12 M sodium phosphate buffer containing 0.22 M  $\text{H}_3\text{BO}_3$ , pH 8.6. After drying, the sheet was marked again with small 'ink dots'.

#### Detection and quantification

The spots on the PEI sheet were localized by autoradiography (0.5–16 h) and quantified by Cerenkov counting after excision of the spots (maximum counting efficiency 43%). The LE for a spot \*pdNp is calculated in percent of the total activity which theoretically could have been incorporated into the dNp. The total radioactivity used was the sum of the activity in the washing water and on the sheet (all spots plus total background). The total background  $\text{BG}_{\text{tot}}$  was determined after development of the second dimension by counting a 1- $\text{cm}^2$  area (~30–150 c.p.m.) on the right-hand side between the phosphate spot and the front, and by multiplying the result by 290 (14.5  $\times$  20  $\text{cm}^2$ ).

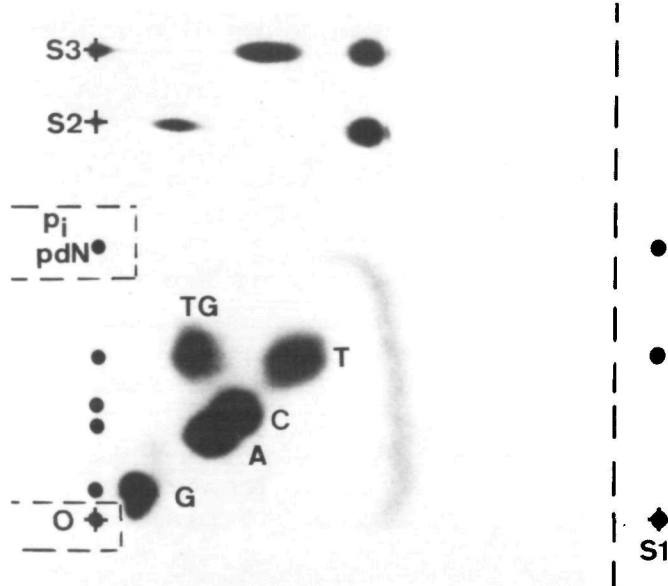


Fig. 1. Two-dimensional TLC on PEI–cellulose for separating bis[5'- $^{32}\text{P}$ ]phosphates of the natural nucleotides (G, A, C and T) and TG. The sample applied was a mixture of two phosphorylation reactions: (i) containing \*pdTGp (from 50 pmol dTGp, pH 7.5, 1 mM  $\text{BeCl}_2$ ); and (ii) containing \*pdNp (from 50 pmol of an equimolar mixture of the four natural nucleotides, phosphorylated at pH 9.0). The small dots indicate the positions of  $\text{P}_i$ , deoxyribonucleoside-5'-monophosphates (pdN), and of all pdNp as seen after the first dimension (developed from bottom to top). Crossed dots indicate the loading areas of the sample (O) and of the standards used in the first (S1) and second dimension (S2 and S3, developed from left to right). Cuts performed after the first dimension are indicated by the interrupted lines.

The amount of nucleotides present during phosphorylation was calculated on the basis of a LE of 10% for dTGp ( $\text{BeCl}_2$ , pH 7.5) and of 80% for normal nucleotides (no  $\text{BeCl}_2$ , pH 9).

## Results

### Purification of dTGp from normal nucleotides

No dTGp fraction collected from the phenylboronate column (nos. 2–7) contained > 10 pmol contaminating dNp after loading 3.2  $\mu\text{mol}$  nucleotide. In the six fractions, therefore, < 1 normal nucleotide/50 000 dNp remains after repetitive PBA chromatography.

The recovery of dTGp chromatographed twice on PBA affinity columns on average was 30% (range 20–40%) as determined by postlabeling. This was irrespective of whether the dTGp (250 pmol) was chromatographed alone or in a digestion mix of 1 mg DNA–hydrolysate (3.2  $\mu\text{mol}$  dNp).

### Postlabeling of dTGp

**With T4 PNK under standard conditions.** With ATP as limiting factor and at pH 8.0, a LE of 1–4% resulted for dTGp and of ~30% for dTp. This was not surprising in view of the fact that dTGp has lost its normal thymine ring structure (loss of the 5,6-double bond).

**Influence of 'mutagenic' divalent cations on the LE of dTGp and dTp at pH 8.0.** A number of divalent metal ions are known to decrease the replicative fidelity of DNA polymerase (10). In order to shift the specificity of the enzymatic phosphorylation reaction of T4 polynucleotide kinase (PNK) to accept thymidine glycol as substrate, different 'mutagenic' divalent cations were used at a concentration of 1 mM.

Addition of  $\text{CdCl}_2$  or  $\text{MnCl}_2$  raised the LE to 5%.  $\text{CoCl}_2$  increased the LE to ~10% for dTGp whereas the LE for dTp

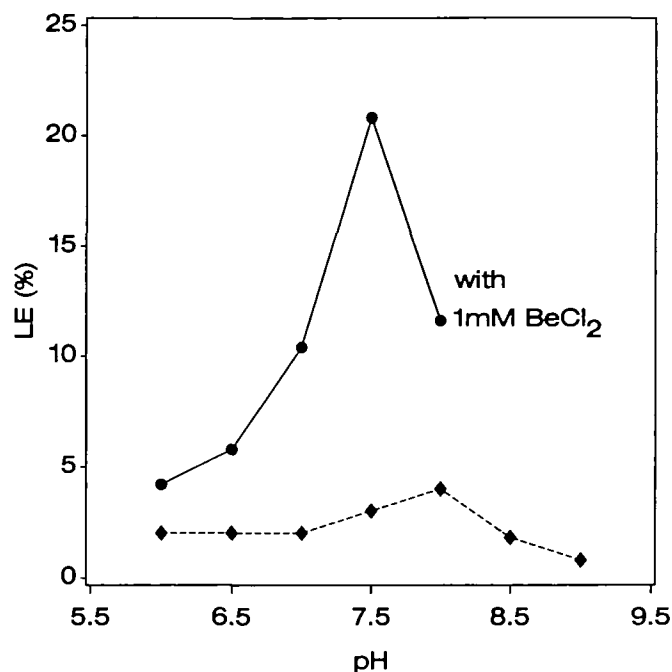


Fig. 2. LE under ATP-deficient conditions as a function of pH of the kinase buffer, for the phosphorylation of thymidine-*cis*-glycol-3'-phosphate (dTGP) in the presence or absence of 1 mM  $\text{BeCl}_2$ .

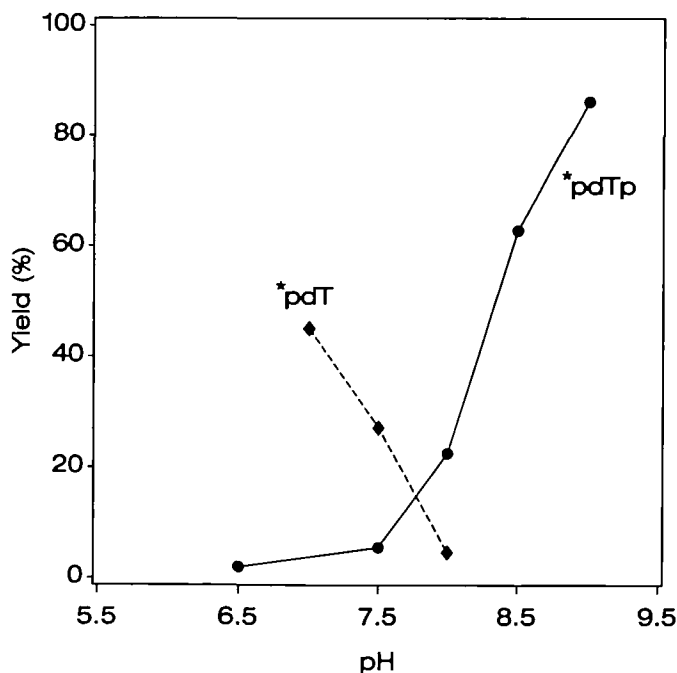


Fig. 3. Phosphorylation of thymidine-3'-phosphate as a function of the pH of the kinase buffer. Solid line, yield of \*pdTp; broken line, yield of 3'-dephosphorylated thymidine \*pdT. \*pdT was determined by developing the TLC with 1 M LiCl, resulting in a separation of \*pdNp, \*p<sub>i</sub> and \*pdN with increasing  $R_f$  values.

did not change appreciably. The best results were obtained in the presence of 1 mM  $\text{BeCl}_2$ : the LE for dTGP increased to up to 20%, whereas the LE for the normal nucleotide dTp decreased to 15%.

**pH dependence of the LE.** In the absence of divalent mutagenic metal ions, the pH optimum for dTGP phosphorylation was at ~8.0. In the presence of 1 mM  $\text{BeCl}_2$ , this optimum shifted to

Table I. LE for the phosphorylation of various amounts of dTGP in the presence or absence of contaminating natural nucleotides (dNp) (performed at >10-fold molar excess of ATP)

Amount of dTGP incubated (pmol)	50	10	1	0.1	0.01
LE (%)					
In the presence of 50 pmol dNp*	14	14	15	No data	
Without dNp added	8	9	9	10	11

\*dNp as equimolar mixture of the four natural DNA constituents.

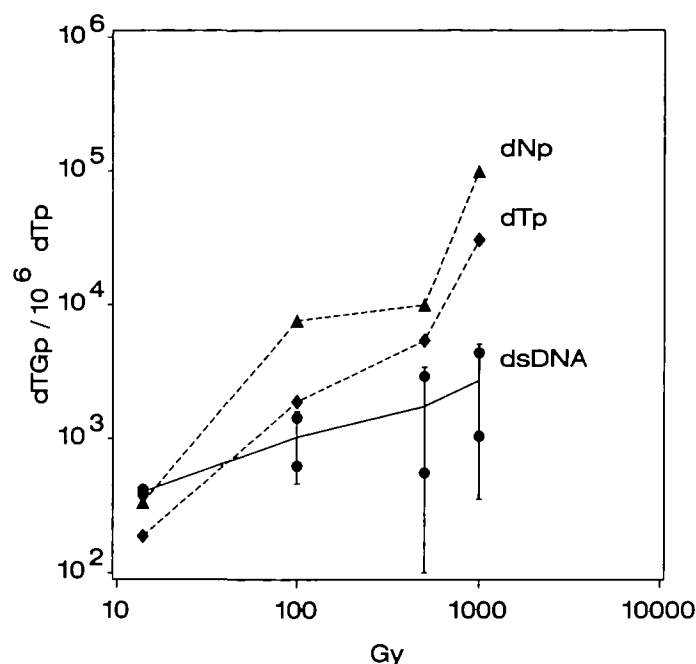
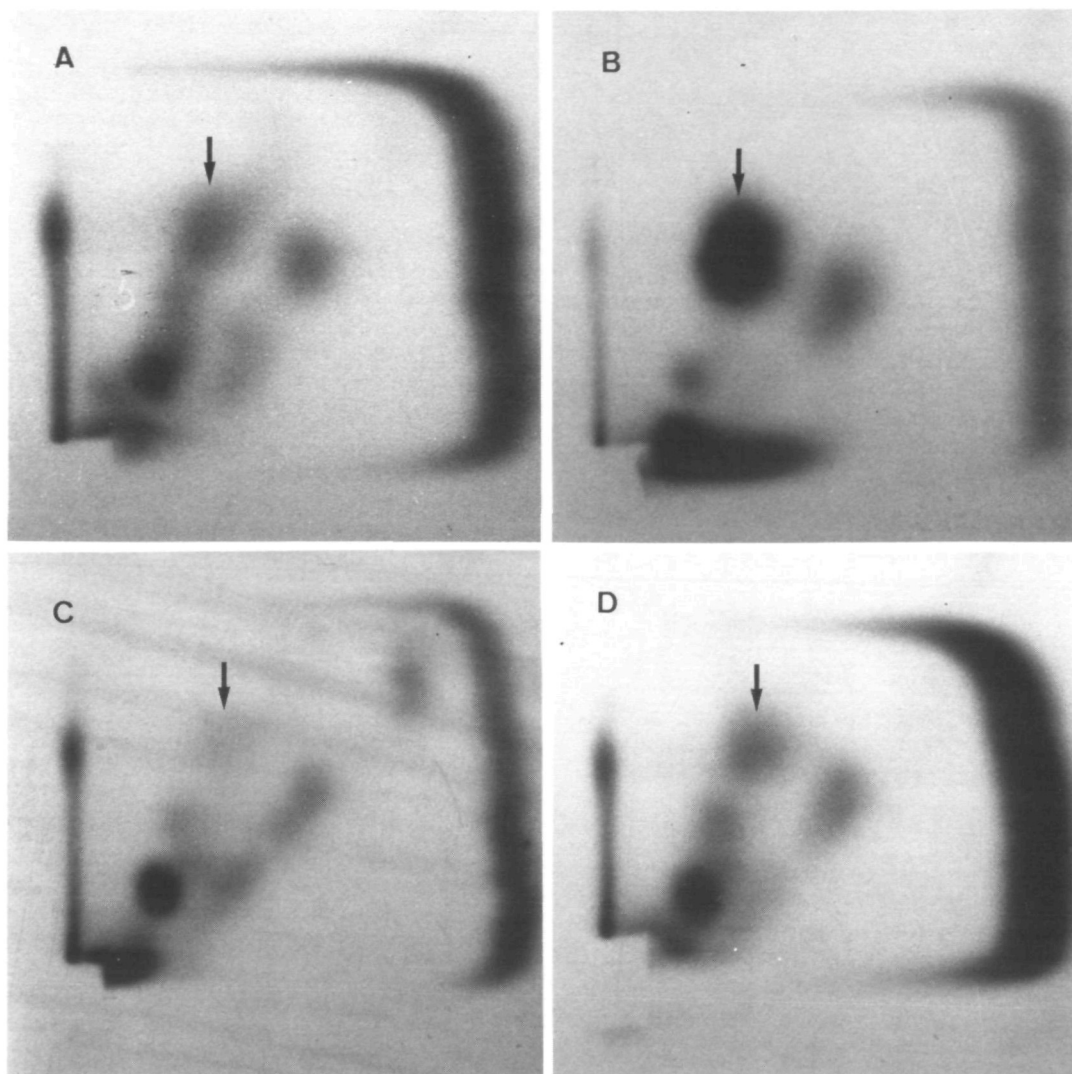


Fig. 4. Formation of thymidine *cis*-glycol as a function of the  $\gamma$ -irradiation energy, expressed per one million thymine residues. Aqueous solutions of dTp, of an equimolar mixture of the four natural nucleotides (dNp) and of commercial calf thymus DNA (in duplicate  $\pm$  1 linear SD) were analysed. Yields of 30 and 10% were used for these calculations to account for the recovery after PBA chromatography and the LE respectively.

pH 7.5 and the LE increased to up to 20% (Figure 2). The normal nucleotide dTp showed a pH optimum at pH 9.0. At lower pH values, the yield of \*pdTp was lower, primarily due to the phosphatase activity of PNK (11). The increasing extent of dephosphorylation in the 3' position resulted in an apparent LE (for forming \*pdTp) at pH 7.5 of only a few percent (Figure 3), in the presence or absence of 1 mM  $\text{BeCl}_2$ .

**Concentration dependence of dTGP phosphorylation.** The LE was investigated over a wide concentration range in the presence of a >10-fold molar excess of ATP (Table I). The dTGP phosphorylation was linear from 50 pmol down to 10 fmol dTGP with a LE of 8–11%. An average value of 10% will be used for all subsequent calculations. In the presence of 50 pmol normal nucleotides the dTGP phosphorylation was still proportional to its concentration and the LE was in the same range as without dNp.

When dTGP was phosphorylated in the presence of contaminating natural nucleotides under the improved conditions, the chemical concentration of ATP had to be at least equimolar to the total amount of dNp + dTGP present. Under ATP-deficient conditions in the presence of natural substrates there was no detectable dTGP phosphorylation.



**Fig. 5.** Two-dimensional TLC on PEI-cellulose for the detection of thymidine *cis*-glycol (as \*pdTGp, indicated by an arrow) after irradiation of nucleotides (A, 14 Gy; B, 100 Gy) and calf thymus DNA (C, 0 Gy = background; D, 14 Gy). Fifty pmol ATP containing 4  $\mu$ Ci  $^{32}$ P was used in these samples for the phosphorylation reaction.

**Additional modifications.** With phosphatase-free PNK (12) dTGp phosphorylation could not be enhanced. Without beryllium the LE was in the same range as with the normal PNK. In the presence of 1 mM  $\text{BeCl}_2$  the LE was even reduced to <1% between pH 6.5 and 8.5.

The use of nuclease P1 was also investigated. Normal nucleotides dNp are dephosphorylated by nuclease P1 to the nucleosides dN which are no longer substrates for the kinase reaction. Since some nucleotide-carcinogen adducts resist dephosphorylation by nuclease P1 (13), it was tested whether dTGp was a substrate or not. Unfortunately, dTGp was dephosphorylated like normal dNp. A LE of only 3% resulted after nuclease P1 treatment of dTGp, with 23% LE in the respective control experiment. This enzyme cannot, therefore, be used for a further improvement of the present method.

#### *Quantification of cis-dTGp formed in irradiated samples*

$\gamma$ -Irradiation of aqueous solutions of thymidine-3'-phosphate, of an equimolar mixture of the four natural nucleotides, and of dsDNA resulted in a dose-dependent increase in the formation of thymidine *cis*-glycol (Figure 4). At the lowest energy used (14 Gy), the level of glycol formation was similar in all samples,

i.e. between 190 and 400 TG/ $10^6$  T. With increasing radiation dose, the formation of TG was proportional to the energy only with thymidine and nucleotides reaching values of 30 000–100 000 TG/ $10^6$  T at 1000 Gy. With DNA, the level of glycol formation increased only to 2700 TG/ $10^6$  T at 1000 Gy. It is possible that highly irradiated DNA accumulates lesions which render it partly resistant to enzymatic hydrolysis to the nucleotides. This hypothesis is supported by the observation that intensity and number of contaminating spots increase with the radiation energy.

Figure 5 shows examples of the two-dimensional TLC. Charts A and B show the chromatograms after irradiation of the nucleotide samples with 14 and 100 Gy. The radioactivity located in the thymidine glycol position (see Figure 1 for reference) markedly increased relative to the contaminating spots. Chart C shows a non-irradiated control DNA. In the location of TG standards, a spot can just be discerned. In this chromatogram, this spot contained 600 c.p.m. This corresponded to the presence of 10 dTGp in  $10^6$  dTp representing the natural background of thymidine glycol in these experiments with commercial calf thymus DNA. Chart D represents DNA after irradiation with 14 Gy. The pdTGp spot is now equivalent in density to the spots

of contaminating products. At higher radiation doses (not shown) the intensity of these spurious spots increased to picomole amounts of substrate.

## Discussion

### The postlabeling method

Since thymidine glycol has lost its normal thymine ring structure it was not surprising to find only low LEs under standard conditions. It was therefore necessary to change the phosphorylation conditions. Firstly the pH was optimized, and secondly it was considered worth trying to use mutagenic metal ions which have been found to decrease the template fidelity of DNA polymerase (10). The aim was to change the T4 PNK specificity so that dTGp is accepted as substrate. Our results show that using beryllium ions and pH 7.5, the glycol is phosphorylated > 10 times more effectively.

Application of the described procedure to non-irradiated calf thymus DNA showed a background of 1 TG/10<sup>5</sup> T. It is not known whether the commercial DNA sample already contained this number of TG or whether the oxidation occurred during work-up. In this sample, a spot was clearly discernible and formed a practical limit of detection. For the determination of an oxidative DNA damage *in vivo*, the limit of detection will depend on this background level of thymidine glycol present in the DNA or formed during work-up. The theoretical limit will be at least one order of magnitude lower when ATP of a higher sp. act. is used. Additional improvements are possible but will require a better quality of the thin-layer system.

### Detection of thymidine glycol as a marker of DNA damage

It was the aim of this study to provide an alternative method for the quantification of one specific type of oxidative DNA damage. The determination of thymine-*cis*-glycols accounts for a sizable fraction of the pyrimidine hydroxylations in DNA arising from ionizing radiation.

The specific reactivity of *cis*-glycols with borate anions was taken advantage of in order to separate thymine glycol from the natural nucleotides. Firstly, PBA affinity chromatography resulted in a > 50 000-fold purification. Secondly, residual contaminating nucleotides were mostly 3'-dephosphorylated and moved with \*p<sub>i</sub> or were finally separated from thymidine glycol by 2-dimensional TLC, with boric acid added to develop the second dimension.

The level of TG formation after  $\gamma$ -irradiation of DNA as determined with the present method fits nicely with the published data using polyclonal antibodies (14), monoclonal antibodies (15) or radiolabeled DNA coupled with chemical separation methods (16). In the dose range between 10 and 100 Gy, our results are always within a factor of 5 compared to all other methods. It therefore seems that the new method represents a sensitive alternative for the determination of a marker damage of oxidized DNA.

## Acknowledgements

This work was supported by the Swiss Science Foundation (grant nos. 3.626-0.84 and 3.041-0.87).

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Received on December 9, 1987; revised on October 2, 1988; accepted on October 4, 1988